INACTIVATION OF *LISTERIA* ON FRANKFURTER SURFACES USING UVC RADIATION AND VACUUM-STEAM-VACUUM PASTEURIZATION.

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Abstract

Listeria monocytogenes, a psychrotrophic food-borne pathogen, is a frequent post-process contaminant on ready-to-eat meat (RTE) products including frankfurters. Both UVC (254 nm) radiation and Vacuum-Steam-Vacuum (VSV) surface steam pasteurization are FDA approved technologies that can be used to decontaminate food surfaces. In this study, the ability of UVC and VSV, alone or in combination, to inactivate *Listeria spp.* on the surfaces of frankfurters that contained antimicrobials, sodium diacetate and potassium lactate, were investigated. UVC radiation, 1 J/cm2 or 2 J/cm2, was able to inactivate between 1.49-1.99 log of *L. monocytogenes* or *L. innocua* on frankfurter surfaces, respectively. In a pilot plant setting VSV (2 cycles, 1.5s, 121C) was able to inactivate 2.56 log of *L. innocua* on frankfurter surfaces. Exposure of frankfurters that were surface-inoculated with *L. innocua* with 1 J/cm^2 UVC prior to VSV treatment resulted in a 3.55 log reduction of *L. innocua*. Combinatorial use of UVC and VSV technologies, in combination with sodium diacetate and potassium lactate, resulted in significant reduction (>99.9%) of *Listeria spp.* on frankfurter surfaces.

Introduction

Listeria monocytogenes is a frequent post-process contaminant of ready-to-eat (RTE) meat products and a number of food-borne illness outbreaks and product recalls have been attributed to *L. monocytogenes*. It is capable of growth at refrigerated temperatures and in high salt environments, which allows it to proliferate during long-term storage (3). Because of the high mortality rate associated with listeriosis, *L. monocytogenes* is given a zero tolerance in ready-to-eat meat products in the United States (2, 7).

Recently, USDA-FSIS (8) has issued regulations regarding testing of RTE meats, and the plants in which they are produced, for contamination with *L. monocytogenes*. Manufacturers which utilize a combination of post-process (cooking) intervention and antimicrobials that result in a 2 log inactivation of *L. monocytogenes*, with subsequent inhibition of *L. monocytogenes* growth over the food's refrigerated shelf-life, would have to meet less stringent Alternative 1 *Listeria* testing requirements. Many manufacturers are therefore interested in combinatorial use of FDA approved intervention technologies and antimicrobials that would help them meet newly issued FSIS Alternative 1 *Listeria* testing requirements for ready-to-eat meats.

UVC radiation (254 nm) is an FDA approved process for surface decontamination of foods (21CFR Part 179). Exposure of bacteria to UVC radiation leads to the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts in their chromosomes, which can result in mutagenesis, DNA strand breakage due to the DNA repair process, blockage of DNA replication, and death of the exposed bacterium. Vacuum-Steam-Vacuum (VSV) technology utilizes alternating cycles of steam and vacuum to inactivate *Listeria* spp. on ready-to-eat meat surfaces. In this study the use of UVC radiation to inactivate *Listeria spp.* on the surfaces of frankfurters that contained sodium diacetate and potassium lactate was investigated. Sodium diacetate and potassium lactate mixtures are GRAS food additives that that are frequently used in frankfurters as *Listeria* growth inhibitors as part of frankfurter formulation (5, 9). Because irradiation of bacteria causes formation of DNA strand breaks as a result of the DNA repair process, which makes bacterial chromosomes heat labile, the use of UVC as a treatment prior to VSV pasteurization to inactivate *Listeria* spp. on frankfurters that contained sodium diacetate and potassium lactate surfaces was also investigated.

Materials and Methods

Frankfurters. Freshly manufactured frankfurters were purchased from a local manufacturer and consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, sodium diacetate (0.07%), potassium lactate (1.13%), sodium erythorbate, and sodium nitrate. Frankfurters were gamma irradiated (10 kGy, -20C) to inactivate background microflora prior to experimentation.

Bacterial Strains. L. monocytogenes strains H7762, H7962, H7969 were obtained from the Centers for Disease Control and Prevention (Atlanta, GA). *Listeria innocua* were obtained from American Type Culture Collection (Manassas, Va) Identity of the isolates was confirmed by Gram Stain, followed by analysis with Gram Positive Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood MO). The bacterial strains were cultured on Tryptic Soy Agar (TSA) (BBL/Difco, Inc., Sparks, MD) at 37°C and maintained at 0-4°C, until use.

UVC Inactivation. The procedure of Sommers and Thayer (2000) for inoculation, irradiation, and enumeration of bacteria was utilized. Each bacterial strain was cultured independently in 25-mL Tryptic Soy Broth (BBL/Difco

Laboratories, Sparks, MD) in a 50-mL conical tube at 37°C (150 rpm) for 18 h. The cells were then pelleted by centrifugation and then resuspended as either a *L. monocytogenes* or *L. innocua* mixture in a total 9-mL of Butterfield's Phosphate Buffer (BPB) (Applied Research Institute, Newtown, CT). Frankfurters were then surface-inoculated with 0.2 mL (10^8 CFU) of *Listeria* and UVC irradiated using a custom made apparatus (10mW/cm2) to obtain the required dose. The frankfurters were washed for 1 min in 100 mL Butterfield's Phosphate Buffer, serially diluted, and pour plated using TSA. Following a 4 hr recovery period at ambient temperature, the bacteria were incubated at 37C for 2 days and the number of colonies per plate enumerated. Each experiment was conducted independently 3 times.

Vacuum-Steam-Vacuum. An Alkar-RapidPak (Lodi, WI) prototype vacuum-steam-vacuum unit was used for the surface steam pasteurization process. Because the Alkar–RapidPak Unit is located in an open air pilot plant, *L. innocua*, as opposed to *L. monocytogenes*, was utilized. Following UVC treatment (1W=1J/s), frankfurters were arranged in single layers (4 frankfurters) within the frankfurter package molds. Frankfurters were then exposed to two cycles of alternating 1.5s steam (121°C) and vacuum treatments. *L. innocua* were recovered and enumerated using standard pour-plate procedures used, as previously described, with the exception that Palcam Medium (BBL/Difco Laboratories, Sparks, MD) was used instead of TSA. Each experiment was conducted independently 3 times.

Results and Discussion

UVC irradiation is an inexpensive FDA approved type of radiation that can be used for the surface decontamination of foods. Previous research has shown that UVC radiation can inactivate 0.5 log reduction of *L. innocua* and *L. monocytogenes* on turkey ham surfaces (unpublished data). Because UV is a non-penetrating type of radiation, bacteria hiding in relatively rough whole muscle surfaces can be shielded from UVC. In contrast to whole muscle ready-to-eat meat surfaces, frankfurter surfaces are relatively smooth (SEM Image, Right). *L. monocytogenes* cells that survive UVC are limited to those in pores and crevices that receive only partial exposure to UVC. A UVC radiation dose of 1.0-2.0 J/cm^2 resulted in a 1.49-1.99 log reduction of *L. monocytogenes* or *L. innocua* on frankfurter surfaces (Table 1).

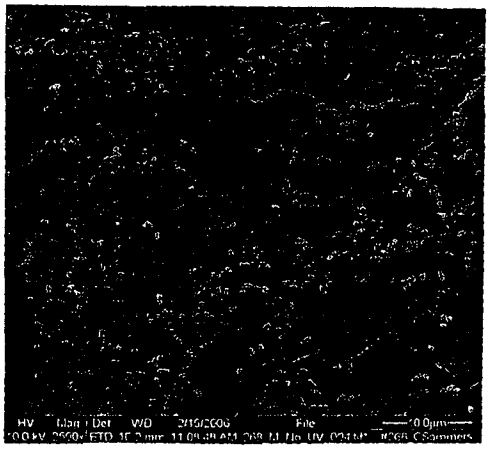

Table 1.
UVC resistance of *Listeria monocytogenes* and *Listeria innocua* surface-inoculated onto frankfurters.

	0 J/cm^2	0.25 J/cm^2	0.5 J/cm^2	1.0 J/cm^2	2.0 J/cm^2
L. monocytogenes	0.00 (±0.00)	-1.10 (±0.12)	-1.31 (±0.06)	-1.49 (±0.07)	-1.93 (±0.13)
L. innocua	0.00 (±0.00)	-1.64 (±0.16)	-1.80 (±0.20)	-1.74 (±0.10)	-1.99 (±0.10)

However, bacteria within pores of frankfurter surfaces may only be partially exposed to UVC radiation. Thayer and Kim (1) have previously shown that gamma radiation induced DNA strand breaks make *Salmonella* more sensitive to thermal processing. Sommers et. al. (6) has demonstrated the same phenomenon for *L. monocytogenes*. Because UVC radiation induces DNA strand breaks and regions of exposed single stranded DNA in the bacterial chromosome due to the DNA repair process and blockage of DNA replication, it was possible that *L. monocytogenes* exposed to UV light could become more sensitive to mild thermal treatment.

Vacuum-Steam-Vacuum (VSV) utilizes alternating cycles of vacuum and steam exposes ready-to-eat meat surfaces to a mild thermal treatment. Sommers et al. (4) demonstrated the use of VSV technology to inactivate 2 log of *L. innocua* on ham skin and ham meat, and 1 log of *L. innocua* on deli turkey meat (unpublished data). Currently in our laboratory *L. innocua* is used as a surrogate for *L. monocytogenes* for the VSV process, as the prototype VSV machine is located in an open air pilot plant. Treatment of frankfurters inoculated with two vacuum and steam

cycles (1.5s, 121C) resulted in a 2.64 log reduction of *L. innocua* in frankfurter surfaces. Use of UV pre-treatment (1 J/cm^2) resulted in an additional inactivation of *L. innocua* for a total 3.55 log reduction (Table 2).

Table 2.
Log reduction of *Listeria innocua* surface-inoculated onto frankfurters following UV and VSV treatment.

	Control	UV	VSV	UV+VSV
L. innocua	0.00 (±0.00)	-1.71 (±0.19)	-2.64 (±0.18)	-3.55 (±0.09)

Use of UVC radiation as a stand alone technology or as a pretreatment prior to application of thermal treatments such as vacuum-steam-vacuum, flash pasteurization, infrared, or in hot water pasteurization, in combination with GRAS antimicrobials, raises interesting possibilities in the quest to inactivate *L. monocytogenes* in ready-to-eat meats and help ready-to-eat meat manufacturers attain FSIS Alternative 1 testing requirement status. Future research will be conducted on the combinatorial use of UVC radiation, mild thermal treatments, and GRAS antimicrobials for that specific purpose.

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